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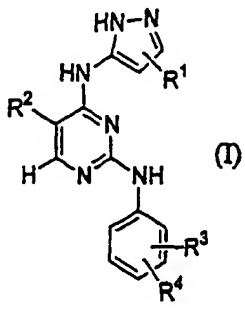
(54) Title: 2-PHENYLAMINO-4-(5-PYRAZOLYLAMINO)-PYRIMIDINE DERIVATIVES AS KINASE INHIBITORS, IN PARTICULAR, SRC KINASE INHIBITORS



(I)

(57) Abstract: This application discloses and claims 5-substituted-2,4-diaminopyrimidines, (I) in which R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, adamantyl, phenyl, or a 5-membered heteroaromatic containing a single heteroatom selected from N, O, and S. R^2 represents H, F, Cl, or C_{1-4} alkyl. R^3 represents H, halogen, $O(C_{¿}1-4alkyl)$, or C_{1-6}alkyl. R?4 represents halogen, NO_2, C_{1-6} alkyl, NR^5R^6, $O(CH_2)_{1-4}$-CO_2R^7, $O(CH_2)_{1-4}$-$C(O)NR^5R^6$, $N(R^5)C(O)CH_2OR^8$, $OC(O)R^9$, $C(O)NR^5R^6$, CO_2R^7, CN, or $O(C_{1-4}$alkyl) optionally substituted by OH or phenoxy, pharmaceutical compositions containing them, a method of making them, and methods of using them for treatment of cancers.

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APPLICATION FOR PATENT

2-PHENYLAMINO-4-(5-PYRAZOLYLAMINO)-PYRIMIDINE DERIVATIVES AS KINASE INHIBITORS, IN PARTICULAR, SRC KINASE INHIBITORS

Field of the Invention

The present invention relates to 5-substituted pyrimidine compounds, and in particular, 2,4-diamine-substituted pyrimidine compounds, and pharmaceutical compositions thereof, and the use of such substituted pyrimidine compounds as inhibitors of src kinase enzymes.

Background of the Invention

Normal tissue homeostasis is achieved by an intricate balance between the rate of cell proliferation and cell death. Disruption of this balance, e.g., by increasing the rate of cell proliferation, modulating the rate of cell differentiation or decreasing the rate of cell death, can result in the abnormal growth of cells and is thought to be a major event in the development of cancer, as well as other cell proliferative disorders such as restenosis.

Proliferative disorders, e.g., cancer, causes significant numbers of deaths. For example, cancer causes over half a million deaths per year in the United States alone. Conventional strategies for the treatment of cancer include chemotherapy, radiotherapy, surgery or combinations thereof, however further advances in these strategies are limited by lack of specificity and excessive toxicity to normal tissues. In addition, certain cancers are refractory to treatments such as chemotherapy, and some of these strategies such as surgery are not always viable alternatives. For example, non-small-cell lung cancer (NSCLC), which includes squamous cell carcinoma, adenocarcinoma and large-cell carcinoma, accounts for 75-80% of all lung cancers (American Cancer Society, 1993). Current multimodality therapeutic strategies applied to regionally advanced NSCLC are minimally effective with the overall cure rate being only about 10% (Belani (1993) Semin Oncol. 20:302 and Roth *et al.* (1994) Lung Cancer 11 Suppl 3:S25).

Cell growth, differentiation and other cell processes are regulated by signal transduction pathways involving protein phosphorylation. Protein phosphorylation is the

result of the transfer of a terminal phosphate of adenosine triphosphate to a particular amino acid of a protein. This transfer is catalyzed by enzymes termed kinases. Protein kinases comprise a large superfamily of homologous proteins. They are related by their kinase or catalytic domains, which consists of approximately 250-300 amino acid residues. There are two main categories within the superfamily of protein kinases: the protein-serine/threonine kinses and the protein-tyrosine kinases (Hanks *et al.*, (1995) FASEB J. 9:576)

Kinases having an abnormal activity, e.g., mutated kinases, or abnormal levels of kinases, have been associated with abnormal cellular processes, which result in specific diseases. For example, several oncogenes, which are capable of transforming cells, are mutated forms of normal genes encoding kinases. Examples of such oncogenes include the pp60-v-src gene from the Rous avian sarcoma virus, which corresponds to the normal (i.e., proto-oncogene) gene pp60-c-src, containing a deletion that removes the C-terminal 18 amino acids of c-src. Pp60-c-src is also referred to as "src kinase" or "src tyrosine kinase." Phosphorylation of a tyrosine residue at position 527 of c-src protein causes a great reduction in its kinase activity, and this site is often altered in oncogenic derivatives of c-src (*see, e.g.*, Brown *et al.*, (1996) Biochem. Biophys. Acta 1287:121). Other proto-oncogenes encoding tyrosine kinases, which when mutated or over-expressed, cause cells to become transformed, include c-yes; c-fps (c-fes); c-abl and c-met. c-abl and c-met are associated with chronic myelogenous leukemia and osteosarcoma, respectively. Proto-oncogenes encoding serine/threonine kinases include c-mos and c-raf (c-mil). Whereas the above-cited proto-oncogenes are intracellular transducers, other proto-oncogenes encode kinases which are cell-surface receptors. Examples of proto-oncogenes encoding cell surface receptors with tyrosine kinase activity include c-fms (or Colony Stimulating Factor -1 (CSF-1) receptor); c-erbB, which is an epidermal growth factor receptor; c-neu (or erbB-2), erbB-3 or erbB-4 which are related to epidermal growth factor receptor; and c-ros, which is related to the insulin receptor.

The role of abnormal kinase activity or protein levels in diseases has been abundantly documented. This has been demonstrated, e.g., by using inhibitors of kinases, in particular tyrosine kinases. Such inhibitors have been shown to be useful for the treatment of disease states characterized by uncontrolled cell proliferation, e.g., cancer, inflammation, psoriasis, pulmonary fibrosis, glomerulonephritis, atherosclerosis, osteoporosis and restenosis following angioplasty. For example, tyrosine kinase inhibitors with selectivity for the EGF

receptor family have been shown to block tumor formation in animals, thus demonstrating their potential usefulness for directly suppressing tumor cell growth in the treatment of human cancer, especially breast carcinoma. Also, tumor metastasis and its associated angiogenesis has been shown to be inhibited by preventing the activation of the vascular endothelial growth factor receptor tyrosine kinase which indicates a utility for tyrosine kinase inhibitors in blocking separate events that occur during carcinogenesis. Thus, protein phosphorylation, e.g., tyrosine phosphorylation, plays an important role in cell regulatory processes, e.g., cell proliferation, and in diseases.

The pp60c-src protein has significant structural homology to about ten proteins (collectively referred to as Src Family kinases or SFKs) which include: Lck, Fyn, Yes, Yrk, Blk, Fgr, Hck, Lyn, and Frk subfamily members Frk/Rak and Iyk/Bsk (Sawyer *et al.*, (2001) Expert Opin. Investig. Drugs 10(7):1327). The Src family of tyrosine kinases, has three major domains: src homology SH1, SH2, and SH3 domains. The SH1 domain is most commonly called the catalytic domain or tyrosine kinase domain. The SH3 domain is a binding region for proteins having proline-rich sequences. Both the SH2 and SH3 domains are noncatalytic, but are important in protein-protein recognition. SH2 domains are homologous motifs of approximately 100 amino acids, which recognize and bind to the phosphorylated sequences present on regulatory proteins and growth factor receptors (Anderson *et al.*, *Science*, 1990, *250*, 979).

One of the primary purposes of the src family phosphoprotein/SH2 domain interaction is to initiate the association of proteins into an activation complex, often around the intracellular domain of the receptor itself. This role of the src family SH2 domain mediates and organizes the ordered, physical assembly of the various proteins in the activation complex. The activity of a number of immunologically important src family SH2 domain-containing proteins, including, Fyn, Fgr, Yes, Lyn, Hck and Lck, is mediated in this way. P56lck is of particular interest because it has been associated with the signal transduction cascade needed for T-cell activation mediated by the T-cell receptor (TCR) (Straus *et al.* (1992) *Cell*, *70*, 585).

The Src family of protein kinases, which all contain an SH2 domain, are involved in a number of cellular signalling pathways. For example, Src is involved in growth factor receptor signaling; integrin-mediated signaling; T- and B-cell activation; osteoclast activation; cell adhesion; cell motility and cell survival. It is known that the Src SH2 domain

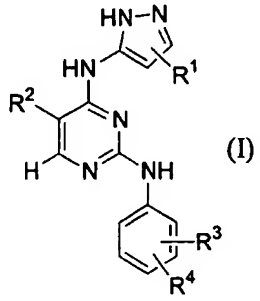
binds to several key receptor and nonreceptor tyrosine kinases such as tyrosine kinases containing receptors for PDGF, EGF, HER2/Neu (an oncogene form of EGF), Fibroblast Growth Factor (FGF), focal adhesion kinase, p130 protein, and p68 protein. In addition, src has been shown to be involved in the regulation of DNA synthesis, mitosis, and other cellular activities (*see, e.g.,* Susa *et al.* (2000) Trends Pharm. Sciences 21:489).

Current cancer therapies utilize a battery of cytotoxic agents and radiation regimens to both decrease and eradicate tumors. The therapeutic index associated with these therapies is narrow and patients suffer from toxic side effects such as hair loss, bone marrow toxicity, loss of intestinal epithelium and mucositis. Many patients derive a therapeutic benefit from such treatment with an initial reduction in tumor mass and stabilization of the disease. However, recurrence is common and many times the tumors acquire a drug resistant phenotype and are refractory to future treatment with chemotherapeutic agents.

The need exists for kinase inhibitors, such as tyrosine kinase inhibitors, that overcome the above-mentioned deficiencies.

Summary of the Invention

In one embodiment, the invention provides compounds for regulating cellular processes involving a kinase such as a tyrosine kinase, in particular, a src kinase. In this aspect, the invention relates to a compound of the formula (I)

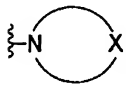


(I)

in which R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, adamantyl, phenyl, or a 5-membered heteroaromatic containing a single heteroatom selected from N, O, and S. R^2 represents H, F, Cl, or C_{1-4} alkyl. R^3 represents H, halogen, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl.

R^4 represents halogen, NO_2, C_{1-6} alkyl, NR^5R^6, $O(CH_2)_{1-4}$-CO_2R^7, $O(CH_2)_{1-4}$-$C(O)NR^5R^6$, $N(R^5)C(O)CH_2OR^8$, $OC(O)R^9$, $C(O)NR^5R^6$, CO_2R^7, CN, or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy. R^5 and R^6 each independently represents H

or C_{1-4} alkyl, or R^5 and R^6 may be joined, and taken together with the nitrogen atom to which they are attached, constitute a 5-6-membered nonaromatic heterocycle



in which X represents NR^5, O, S, or $C(R^5)_2$. R^7 represents H, C_{1-6} alkyl, or phenyl. R^8 represents H, phenyl, benzyl, or C_{1-6} alkyl. R^9 represents C_{1-6} alkyl or phenyl.

In addition, R^3 and R^4 may be joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5 .

Pharmaceutically acceptable salt are also within the scope of the invention.

In another aspect, the invention relates to a pharmaceutical composition comprising a compound of formula (I) as described above, and a pharmaceutically acceptable carrier.

In yet another embodiment, the invention provides methods for regulating cellular processes involving a kinase, such as a tyrosine kinase. In a preferred embodiment, the cellular process involves a src kinase. The cellular process can be, e.g., cell proliferation or cell differentiation.

The invention provides methods for treating diseases associated with a kinase, e.g., diseases associated with an abnormal kinase activity or level, such as cancers, osteoporosis, and inflammatory disorders. The invention also provides methods for treating diseases associated with abnormal cell proliferation and/or differentiation. In a preferred embodiment, the method comprises administering to a subject in need thereof, a pharmaceutically efficient amount of a compound of the invention, such that the subject is treated.

The invention also provides methods for preparing the compounds of the present invention. Also within the scope of the invention are kits comprising one or more compounds of the invention, optionally in a pharmaceutical composition.

Detailed Description of the Invention

The invention is based at least in part on the observation that 2,4-diamino substituted pyrimidine compounds inhibit the activity of src kinases. Exemplary compounds are

described herein.

In formula (I), R^1 is preferably C_{1-6} alkyl, C_{3-6} cycloalkyl, or phenyl; more preferably C_{1-6} alkyl or C_{3-6} cycloalkyl; and most preferably C_{1-6} alkyl. R^2 is preferably H or F. R^3 is preferably H, Cl, F, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl; more preferably H, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl; and most preferably H or $O(C_{1-4}$ alkyl).

R^4 is preferably halogen, NO_2, C_{1-6} alkyl, NR^5R^6, $O(CH_2)_{1-4}$-CO_2R^7, $N(R^5)C(O)CH_2OR^8$, $OC(O)R^9$, $C(O)NR^5R^6$ or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy; it is more preferably C_{1-6} alkyl, NR^5R^6, $N(R^5)C(O)CH_2OR^8$, $C(O)NR^5R^6$ or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy; and it is most preferably NR^5R^6, $N(R^5)C(O)CH_2OR^8$ or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy. The groups R^5 through R^9 have been defined above in the description of formula (I).

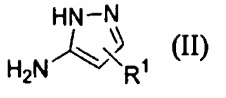
R^3 and R^4 may also preferably be joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from the group consisting of O, S, S(O), $S(O)_2$, and NR^5.

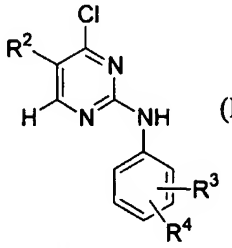
The compounds of the invention have been broadly defined in the summary above and in claim 1. In a preferred embodiment, the compounds of the invention are described by formula (I) in which R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, or phenyl; R^2 represents H, Cl, F, or C_{1-4} alkyl; R^3 represents H, Cl, F, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl; R^4 represents halogen, NO_2, C_{1-6} alkyl, NR^5R^6, $O(CH_2)_{1-4}$-CO_2R^7, $N(R^5)C(O)CH_2OR^8$, $OC(O)R^9$, $C(O)NR^5R^6$, or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy; and R^3 and R^4 are joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5.

In a more preferred embodiment, the compounds of the invention are described by formula (I) in which R^1 represents C_{1-6} alkyl or C_{3-6} cycloalkyl; R^2 represents H or F; R^3 represents H, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl; and R^4 represents C_{1-6} alkyl, NR^5R^6, $N(R^5)C(O)CH_2OR^8$, $C(O)NR^5R^6$ or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy.

In a most preferred embodiment, the compounds of the invention are described by formula (I) in which R^1 represents C_{1-6} alkyl; R^2 represents H or F; R^3 represents H or $O(C_{1-4}$ alkyl); and R^4 represents NR^5R^6, $N(R^5)C(O)CH_2OR^8$ or $O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy.

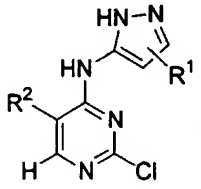
The compounds of formula (I) are generally made by coupling a compound of formula (II)

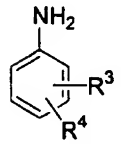

(II) with a compound of formula (III)


(III)

or

coupling a compound of formula (IV)


(IV) with a compound of formula (V)


(V)

, to produce a compound of formula (I).

In formulae (II), (III), (IV), and (V), the meanings of the substituent groups R^1, R^2, R^3, and R^4 are as described above.

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The terms "a" and "an" refer to "one or more" when used in this application, including the claims.

"Abnormal growth of cells" means cell growth independent of normal regulatory mechanisms (e.g., loss of contact inhibition).

The term "analog" of a compound refers to a compound having a substantial structural similarity to a particular compound and having essentially the same type of biological activity as the compound.

The term "antiproliferative" therapeutic or compound refers to a compound or therapeutic which inhibits cell proliferation to at least some extent.

The term "cytostatic" when referring to the activity of a compound means that the compound causes the cell to cell cycle arrest, but it does not kill the cell. Thus, removal of the drug from the environment of the cell results in the resumption of cell proliferation.

The term "derivative" of a compound or of a small molecule refers to a compound which can be derived, e.g., by chemical synthesis, from the original compound. Thus a derivative of a compound has certain structural similarities with the compound.

"Disease associated with an abnormal activity or level of a kinase" refers to a disease in which an abnormal activity or protein level of a kinase is present in certain cells, and in which the abnormal activity or protein level of the kinase is at least partly responsible for the disease.

A "disease associated with a kinase" refers to a disease that can be treated with a kinase inhibitor.

"Diseases associated with src kinase-mediated signaling" refers to diseases which can be treated with an inhibitor of src kinase-mediated signaling. Such disease can, e.g., be associated with an abnormal src kinase activity or level.

The terms "excessive cell proliferation," used interchangeably herein with "hyper-proliferation" of cells refers to cells which divide more often than their normal or wild-type counterpart. Thus, cells are excessively proliferating when they double in less than 24 hours if their normal counterparts double in 24 hours. Excessive proliferation can be detected by simple counting of the cells, with or without specific dyes, or by detecting DNA replication or transcription, such as by measuring incorporation of a labeled molecule or atom into DNA or RNA.

"Inhibiting cell proliferation" refers to decreasing the rate of cell division, by interrupting or slowing down the cell cycle. The term refers to complete blockage of cell proliferation, i.e., cell cycle arrest, as well as to a lengthening of the cell cycle. For example, the period of a cell cycle can be increased by about 10%, about 20%, about 30, 40, 50, or 100%. The duration of the cell cycle can also be augmented by a factor of two, three, 4, 5, 10 or more.

"Modulating cell differentiation" refers to the stimulation or inhibition of cell differentiation.

"Normalizing cell proliferation" refers to reducing the rate of cell proliferation of a cell that proliferates excessively relative to that of its normal or wild-type counterpart, or

increasing the rate of cell proliferation of a cell that proliferates poorly relative to its normal or wild-type counterpart.

A "patient" or "subject" to be treated by the subject method can mean either a human or non-human animal.

The term "proliferative disorder" refers to any disease/disorder of a tissue marked by unwanted or aberrant proliferation of at least some cells in the tissue. Such diseases include cancer, as well as benign diseases or disorders, such as warts or other benign tumors.

A "src inhibitor" is a compound which inhibits at least part of the activity of a src kinase in a cell. The inhibition can be, at least about 20%, preferably at least about 40%, even more preferably at least about 50%, 70%, 80%, 90%, 95%, and most preferably at least about 98% of the activity of the src kinase.

"Treating" a disease refers to preventing, curing or improving at least one symptom of a disease.

The following definitions pertain to the structure of the compounds:

The abbreviations Me, Et, and Ph, represent methyl, ethyl, and phenyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry* (i.e. *J. Org. Chem.* **1995**, *60*, 12a.). This list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in this list are hereby incorporated by reference.

"Alkyl" means a hydrocarbon radical having up to a maximum of 12 carbon atoms, which may be linear or branched with single or multiple branching. Alkyl is especially lower alkyl. Examples of such alkyl groups are methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, hexyl, and isohexyl.

"Halogen" means fluorine, chlorine, bromine, or iodine but is especially fluorine, chlorine, or bromine.

"Cycloalkyl" is a saturated carbocycle that contains between 3 and 12 carbons but preferably 3 to 8 carbons. Examples include the cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl groups.

The term "optionally" means that the subsequent desribed event(s) may or may not occur, and includes both event(s), which occur, and event(s) that do not occur.

Examples of "5-membered heteroaromatic containing a single heteroatom selected

from N, O, and S" include, but are not limited to, furanyl, pyrrolyl, and thienyl.

Examples of "5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5" include, but are not limited to, 1,3-dioxolanyl, 1,4-dioxanyl, tetrahydrofuranyl, piperdinyl, imidazolidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, and piperidinyl.

Abbreviations and Acronyms

When the following abbreviations are used throughout the disclosure, they have the follow meaning:

ATP	adenosine triphosphate
Ar	argon
BRIJ	polyoxyethylene(23) lauryl ether
BSA	bovine serum albumin
n-BuOH	1-butanol
CD_3OD	methanol-d_4
$CDCl_3$	chloroform-*d*
CH_2Cl_2	methylene chloride
CH_3CN	acetonitrile
DMF	*N,N*-Dimethylformamide
DMSO	dimethylsulfoxide
EDTA	ethylencdiaminetetraacetic acid
ESI-MS	electrospray ionization mass spectrometry
EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
H_2	hydrogen gas
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid
Hex	hexanes
^1H NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography

KOAc	potassium acetate
LC/MS	liquid chromatography / mass spectrometry
MeOH	methanol
$MgSO_4$	anhydrous magnesium sulfate
MMTV	murine mammary tumor virus
MS ES	mass spectroscopy with electrospray
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
Poly-GAT	poly glycine, alanine, tyrosine
RNA	ribonucleic acid
Streptavidin-APC	steptavadin conjugated allopycocyanin
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Compounds of the Invention

The present invention provides substituted pyrimidine compounds, e.g., 2,4-diamino substituted pyrimidine compounds, which are capable of inhibiting src kinase activity. The compounds of the invention have the IUPAC names set forth below:

Example #	IUPAC NAME
1	3-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]-1,2-propanediol
2	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-methylphenyl)-2,4-pyrimidinediamine
3	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-nitrophenyl)-2,4-pyrimidinediamine
4	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-chlorophenyl)-2,4-pyrimidinediamine

5	N^2-(4-bromophenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-2,4-pyrimidinediamine
6	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-5-fluoro-N^2-(3-nitrophenyl)-2,4-pyrimidinediamine
7	N^2-(3-bromophenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-2,4-pyrimidinediamine
8	N^2-(3-bromophenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-5-fluoro-2,4-pyrimidinediamine
9	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-[3-(dimethylamino)phenyl]-2,4-pyrimidinediamine
10	2-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]ethanol
11	*tert*-butyl [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenoxy]acetate
12	*tert*-butyl [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]acetate
13	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-[3-(2-phenoxyethoxy)phenyl]-2,4-pyrimidinediamine
14	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-ethylphenyl)-5-fluoro-2,4-pyrimidinediamine
15	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-ethylphenyl)-2,4-pyrimidinediamine
16	N^2-(3-*tert*-butylphenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-5-fluoro-2,4-pyrimidinediamine
17	N^2-(3-*tert*-butylphenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-2,4-pyrimidinediamine
18	2-[5-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)-2-methoxyphenoxy]ethanol
19	2-[5-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)-2-chlorophenoxy]ethanol

20	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-ethoxyphenyl)-2,4-pyrimidinediamine
21	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-methoxyphenyl)-2,4-pyrimidinediamine
22	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(3-isopropoxyphenyl)-2,4-pyrimidinediamine
23	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-(2,3-dihydro-1-benzofuran-5-yl)-2,4-pyrimidinediamine
24	2-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]-*N,N*-diethylacetamide
25	2-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenoxy]-*N,N*-diethylacetamide
26	N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-N^2-{3-[2-(4-morpholinyl)-2-oxoethoxy]phenyl}-2,4-pyrimidinediamine
27	N^2-(3-aminophenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-5-fluoro-2,4-pyrimidinediamine
28	N^2-(3-aminophenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-2,4-pyrimidinediamine
29	2-(benzyloxy)-*N*-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenyl]acetamide
30	*N*-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenyl]-2-phenoxyacetamide
31	*N*-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenyl]benzamide
32	2-(benzyloxy)-*N*-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenyl]acetamide
33	*N*-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenyl]-2-phenoxyacetamide
34	[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenoxy]acetic acid

35	butyl [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]acetate

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivatization with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group such as amino, or an acidic functional group such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., functioning as src kinase inhibitors), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound in inhibiting src kinases.

In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures known to those skilled in the art. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

Methods of use of the compounds of the invention

The compounds of the invention, including substituted pyrimidine compounds, salts, prodrugs, and compositions thereof, can be used for treating a disease or condition (generally

referred to herein as " disease") associated with a kinase, such as a disease associated with an abnormal activity or level of a kinase. In a preferred embodiment, the kinase is a tyrosine kinase, such as a src tyrosine kinase. Generally, the compounds of the invention can be used for treating diseases that are associated with a component of the signal transduction pathway in which a kinase is involved. For example, it is expected that a cell proliferative disease resulting from over-expression of a signal transduction molecule or cell surface receptor that is in the same signal transduction pathway as that in which a kinase which can be inhibited by a compound of the invention is present, can also be treated with the compounds of the invention. At least for this reason, the compounds of the invention are expected to be effective against a broad range of target cells, and not only target cells having an abnormal activity or level of a kinase. The terms "target cell" refers to a cell towards which a compound is targeted. Furthermore, at least some of the compounds of the invention may also be effective against cells which proliferate and/or differentiate normally, i.e., wild-type cells. For example, certain compounds could be used to arrest cell proliferation, even if the cell proliferation is not abnormal.

In a preferred embodiment, the compounds of the invention are useful for treating a disease associated with a src kinase. Src kinases are involved in various cellular functions, including cell proliferation and transformation; cell adhesion, migration and chemotaxis; intracellular trafficking; and cell survival. Accordingly, diseases that can be treated according to the invention include those which are dysfunctional in any of these cellular functions. Exemplary diseases are provided below.

In one embodiment, a therapeutic method comprises administering to a subject having a disease associated with a kinase, a pharmaceutically effective amount of a compound of the invention, such that the disease is treated. The subject is preferably a mammal, e.g., a human, non-human primate, bovine, ovine, porcine, feline, canine, mouse or rat. The compounds can be administered via various routes depending on the disease to be treated. Methods of administration are further described herein. Non-mammalian cells, which share essentially the same signal transduction pathways as those in mammalian cells, e.g., yeast cells, can also be target cells of the invention.

Compounds of the invention may specifically inhibit the activity of a single kinase, e.g., src kinase, or they may inhibit the activity of more than one kinase or more than one

type of kinase. Accordingly, a compound of the invention could be used for treating one or more diseases associated with one or more kinases.

The efficacy of the compounds of the invention against a broad range of target cells allows for broad applications for these compounds. The following are exemplary therapeutic applications for the compounds of the invention. These exemplary therapeutic applications focus first on diseases associated with src tyrosine kinase and then describe other diseases that may also be treated with the compounds of the invention.

Src tyrosine kinase has specifically been implicated in the development, growth, progression, and metastasis of a number of human cancers such as colon, breast, pancreas and brain (*see, e.g.,* Irby and Yeatman (2000) Oncogene 19:5636), and these cancers are expected to be treatable with the compounds of the invention. For example, a src kinase activity from 4-20 fold higher than normal has been found in mammary carcinomas (Irby and Yeatman, *supra*; Egan *et al.* (1999) Oncogene 18:1227 and Verbeek *et al.* (1996) J. Pathol. 180:383).

c-src has also frequently been implicated in the initiation and progression of human colon cancer and in resultant metastases (*see, e.g.,* Cartwright *et al.* (1994) J. Clin. Invest. 93:509; Talamonti *et al.* (1991) J. Clin. Invest. 91:53; and Termuhlen *et al.* (1993) J. Surg. Res. 54). Src is increased 5-8 fold in the majority of colon tumors. Elevated src activity is also present in pre-cancerous colon lesions, e.g., adenomatous polyps (Pena *et al.* (1995) Gastroenterol. 108:117).

Other cancers that can be treated include pancreatic cancer (Flossmann-Kast *et al.* (1998) Cancer Res. 8:3551); and Visser *et al.* (1996) Lab. Invest. 74:2), lung cancer (Mazurenko *et al.* (1992) Eur. J. Cancer 28:372), neural cancer (Bjelfman *et al.* (1990) Cancer Res. 50:6908); ovarian cancer (Wiener *et al.* (1999) Clin. Cancer Res. 5:2164); esophageal adenocarcinomas and Barrett's (Kumble *et al.* (1997) Gastroeneterology 112:348); gastic cancers (Takeshima *et al.* (1991) Jpn. J. Cancer Res. 82:1428); melanomas (Bjorge *et al.* (1996) Biochem. Cell Biol. 74:477) and Kaposi's sarcoma (Munshi *et al.* (2000) J. Immunol. 164:1169). Src probably also contributes to tumor growth in synergy with receptor tyrosine kinases, such as c-met and those of the ErbB family (Biscardi *et al.* (1999) Adv. Cancer Res. 76: 6). Accordingly, all of the above are exemplary cancers that can be treated with the compounds of the invention.

The compounds of the invention can also be used to treat diseases associated with defects in cell adhesion and motility, such as angiogenesis, inflammation and bone resorption. Src has been shown to play a role in signal transduction via cell-adhesion receptors (integrins). Src dependent cell migration is important for the function of many cell types, e.g., the motility of osteoclasts and metastasizing cells (Chellaiah *et al.* (2000) J. Biol. Chem. 275:11993 and Susa and Teri (2000) Drug News Perpect. 13:169). Src dependent cell migration may also be important for the recruitment of vascular smooth muscle cell precursors in response to PDGF produced by endothelial cells during blood vessel formation (Hirschi *et al.* (1998) J. Cell. Biol. 141:805).

Src kinase is also involved in endocytosis, e.g., transcytosis, such as that which occurs in osteoclasts (Nesbitt and Horton (1997) Science 276:266). Src assists endocytosis of certain growth factor receptors, e.g., EGF receptors (Wilde *et al.* (1999) Cell 96:677). Blood vessel hyperpermeability induced by vascular endothelial growth factor (VEGF) is also dependent on src (Eliceiri *et al.* (1999) Mol. Cell 4:915). Src has been shown to also be involved in cell survival (reviewed in Susa *et al.* (2000) Trends in Pharmacol. Sci. 21:489). Accordingly, diseases related to any of these exemplary src biological activities can be treated with the compounds of the invention.

A preferred use for the compounds of the invention is for the treatment of osteoporosis, which involves bone resorption. Osteoporosis is a widespread disease of low bone mass that particularly affects post-menopausal women (*see, e.g.*, Gowen *et al.* (2000) Emerging Drugs 5:1). The role of src in bone metabolism was first demonstrated in src-deficient mice and has been confirmed using small molecular weight inhibitors in animal models of osteoporosis. Src-deficient mice have defective bone resorption, resulting in excessive bone mass and osteopetrosis (*see, e.g.*, Thomas and Brugge (1997) Annu. Rev. Cell. Dev. Biol., 13: 513). The role of src in bone resorption is well recognized. A src inhibitor has been shown to reduce bone resorption in an animal model of osteoporosis (Missbach *et al.* (1992) Bone 24:437). The disorder is believed to be caused by dysfunctions in osteoclasts and osteoblasts, as well as in osteoclast survival and osteoclast formation (reviewed in Susa *et al.*, *supra*).

Other diseases that may also be treated according to the invention include other types of malignancies, e.g., cancers of the brain, genitourinary tract, prostate, skin, lymphatic system, rectum, stomach, larynx, ovary, bladder, and liver. More particularly, such cancers

include histiocytic lymphoma, lung adenocarcinoma, pancreatic carcinoma, colo-rectal carcinoma, bladder cancers, head and neck cancers, acute and chronic leukemias, melanomas, neurological tumor, myeloid leukemias (for example, acute myelogenousleukemia), sarcomas, thyroid follicular cancer, and myelodysplastic syndrome.

The compounds of the invention can also be used for treating disease associated with abnormal activity and/or expression of members of a growth factor family or receptors thereof. For example, compounds of the invention are expected to be effective against diseases associated with a defect in a growth factor or receptor of the EGF receptor family, such as Neu-erb2-related genes. The compounds of the invention are believed to be effective against the following diseases. For example, amplification and/or over-expression of human erbB2 gene, has been shown to correlate with a poor prognosis in breast and ovarian cancers, in particular, carcinomas (*see, e.g.,* Slamon *et al.*, Science 235:177-82 (1987); Slamon *et al.*, Science 244:707-12 (1989)). Overexpression of erbB2 has also been correlated with other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon and bladder. ErbB1 has been causally implicated in human malignancy, e.g., aggressive carcinomas of the breast, bladder, lung, and stomach. ErbB gene amplification or overexpression, or a combination of both, has also been demonstrated in squamous cell carcinomas and glioblastomas (Libermann, T. A., Nusbaum, H. R., Razon, N., Kris, R., Lax, I., Soreq, H., Whittle, N., Waterfield, M.D., Ullrich, A. & Schlessinger, J., 1985, Nature 313:144-147). Accordingly, the compounds of the invention are believed to be useful for treating these malignancies. ErbB3 has been found to be overexpressed in breast (Lemoine *et al.*, Br. J. Cancer 66:1116-21 (1992)), gastrointestinal (Poller *et al.*, J. Pathol. 168:275-80 (1992); Rajkumer *et al.*, J. Pathol. 170:271-78 (1993); Sanidas *et al.*, Int. J. Cancer 54:935-40 (1993)), and pancreatic cancers (Lemoine *et al.*, J. Pathol. 168:269-73 (1992), and Friess *et al.*, Clinical Cancer Research 1:1413-20 (1995)). Plowman *et al.* found that Increased erbB4 expression have been found to closely correlate with certain carcinomas of epithelial origin, including breast adenocarcinomas (Plowman *et al.*, PNAS 90:1746-50 (1993) and Plowman *et al.*, Nature 366:473-75 (1993)).

The hyper-proliferative disorders that can be treated by the disclosed substituted pyrimidine compounds, salts, prodrugs and compositions thereof include, but are not limited to solid tumors, such as cancers of the breast, respiratory tract, brain, reproductive organs, digestive tract, urinary tract, eye, liver, skin, head and neck, thyroid, parathyroid and their

distant metastases. Those disorders also include, but are not limited to lymphomas, sarcomas, and leukemias.

Examples of breast cancer include, but are not limited to invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ.

Examples of cancers of the respiratory tract include, but are not limited to small-cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma.

Examples of brain cancers include, but are not limited to brain stem and hypophtalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, as well as neuroectodermal and pineal tumor.

Tumors of the male reproductive organs include, but are not limited to prostate and testicular cancer.

Tumors of the female reproductive organs include, but are not limited to endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus.

Tumors of the digestive tract include, but are not limited to anal, colon, colorectal, esophageal, gallblader, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers.

Tumors of the urinary tract include, but are not limited to bladder, penile, kidney, renal pelvis, ureter, and urethral cancers.

Eye cancers include, but are not limited to intraocular melanoma and retinoblastoma.

Examples of liver cancers include, but are not limited to hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma.

Skin cancers include, but are not limited to squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer.

Head-and-neck cancers include, but are not limited to laryngeal / hypopharyngeal / nasopharyngeal / oropharyngeal cancer, and lip and oral cavity cancer. Lymphomas include, but are not limited to AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Hodgkin's disease, and lymphoma of the central nervous system.

Sarcomas include, but are not limited to sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma. Leukemias include, but are not limited to acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia.

These disorders have been well characterized in man, but also exist with a similar etiology in other mammals, and can be treated by pharmaceutical compositions of the present invention.

Other types of proliferative disorders that can be treated according to the invention include non malignant cell proliferative disorders, such as those associated with an abnormal production of, or response to a growth factor, e.g., platelet derived growth factor (PDGF), fibroblast derived growth factor (FGF), epidermal derived growth factor (EGF) and vascular endothelial growth factor (VEGF). Exemplary diseases include restinosis, glomerulonephritis, neurofibromatosis, glaucoma, psoriasis, rheumatoid arthritis, inflammatory bowel disease, and chemotherapy-induced alopecia and mucositis.

Restenosis following coronary angioplasty is one major unsolved problem of interventional cardiology. Of the nearly 400,000 angioplasties currently performed in the United States each year, 25-34% fail within the first five years, of which most occur during the first year, due to restenosis (Geschwind H.J. (1995) Interv. Cardiol. 8:756 and The Merck Manual of Diagnosis and Therapy, 16th Ed. (1992) Merck Res. Lab., p. 406. The process of restenosis involves the reocclusion of an atherosclerotic artery which in many cases is due to the proliferation of smooth muscle cells which is mediated by growth factors such as PDGF and FGF. In animal models of restenosis, antibodies which block the activation of PDGF or FGF receptor tyrosine kinase activity prevent smooth muscle cell proliferation and the formation of neointima. These studies indicate that tyrosine kinase inhibitors that block PDGF or FGF receptor function could have utility in treating human restenosis.

In experimental models of glomerulonephritis, a 20-fold increase in PDGFR expression is associated with mesangial cell proliferation. Neutralization of PDGF which prevents the activation of its tyrosine kinase receptor limits the amount of renal degeneration which normally occurs. These studies demonstrate that a tyrosine kinase inhibitor which blocks PDGFR could have potential for the treatment of human glomerulonephritis. Johnson *et al.* (1992) J. Exp. Med. 175:1413.

In another embodiment, the compounds of the invention are used for treating inflammatory diseases, e.g., rheumatoid arthritis (R.A.). Synovial tissues of RA patients express high levels of FGF and PDGF compared with synovial tissues of osteoarthritis patients, a non invasive joint disease (Sano *et al.*, J. Cell. Biol. 110:1417-1426, 1990). These data are consistent with the theory that PDGF and FGF play a role in generating an invasive

tumor-like behavior in arthritic joints of RA synovial connective tissues (Sano *et al.*, J. Clin. Invest. 91:553-565 1993).

It is further expected that the compounds of the invention are useful for treating smooth muscle cell hyper-proliferation, at least in part since PDGF is considered to be a principal growth-regulatory molecule responsible for smooth muscle cell proliferation. One smooth muscle disorder is atherosclerosis, which is a disease characterized by focal thickening of the inner portion of the artery wall, predisposing an individual to myocardial infarction (heart attack), cerebral infarction (stroke), hypertension (high blood pressure) and gangrene of the extremities. In addition to consisting primarily of proliferated smooth muscle cells, lesions of atherosclerosis are surrounded by large amounts of lipid-laden macrophages, varying numbers of lymphocytes and large amounts of connective tissue. PDGF has been found in numerous cells in such lesions, and it is believed that PDGF plays a critical role in the atherosclerosis disease process. Other smooth muscle diseases include diabetic vascular pathologies.

Both FGF and VEGF are potent angiogenic factors that induce formation of new capillary blood vessels. Accordingly, the compounds of the invention may be useful in inhibiting vascularization, e.g., in tumors.

In addition, the instant compounds may also be useful in the treatment of certain viral infections, in particular in the treatment of hepatitis C or delta and related viruses (Glenn *et al.* Science, 256:1331-1333 (1992)). Numerous viruses also induce non cancerous cell proliferation. Examples include papilloma viruses (HPV), which create skin lesions. Such viral infections may also be treatable with the compositions of the invention.

The compounds of the invention can also be used for treatment of hyperproliferative cutaneous diseases, e.g., keratosis and psoriasis.

Also within the scope of the invention are methods for inhibiting growth of non-mammalian cells, which have similar signal transduction pathways as those in mammalian cells. Exemplary cells include yeast cells. Accordingly, the compounds of the invention can be used as anti-fungal agents to treat fungal infections on animals, e.g., humans. The compounds can also be used for stopping fungus growth on objects, e.g., mold or mildew growth on shower curtains.

A person of skill in the art would understand, based on the instant description, that other diseases can also be treated according to the invention.

Description of the Pharmaceutical Compositions and Methods of Administration of the Compounds of the Invention

Pharmaceutically acceptable salts of these compounds as well as commonly used prodrugs of these compounds are also within the scope of the invention.

Salts, especially pharmaceutically acceptable salts, of the compounds of the invention such as, for example, organic or inorganic acid addition salts, are also provided by the invention. Suitable inorganic acids include but are not limited to halogen acids (such as hydrochloric acid), sulfuric acid, or phosphoric acid. Suitable organic acids include but are not limited to carboxylic, phosphonic, sulfonic, or sulfamic acids, with examples including acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, 2- or 3-hydroxybutyric acid, γ-aminobutyric acid (GABA), gluconic acid, glucosemonocarboxylic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azeiaic acid, malic acid, tartaric acid, citric acid, glucaric acid, galactaric acid, amino acids (such as glutamic acid, aspartic acid, N-methylglycine, acetytaminoacetic acid, N-acetylasparagine or N-acetylcysteine), pyruvic acid, acetoacetic acid, phosphoserine, and 2- or 3-glycerophosphoric acid.

Formation of prodrugs is well known in the art in order to enhance the properties of the parent compound; such properties include solubility, absorption, biostability and release time (see *"Pharmaceutical Dosage Form and Drug Delivery Systems"* (Sixth Edition), edited by Ansel *et al.*, publ. by Williams & Wilkins, pgs. 27-29, (1995)). Commonly used prodrugs of the disclosed 2,4-diamino-pyrimidine compounds can be designed to take advantage of the major drug biotransformation reactions and are also to be considered within the scope of the invention. Major drug biotransformation reactions include N-dealkylation, O-dealkylation, aliphatic hydroxylation, aromatic hydroxylation, N-oxidation, S-oxidation, deamination, hydrolysis reactions, glucuronidation, sulfation and acetylation (see *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Ninth Edition), editor Molinoff *et al.*, publ. by McGraw-Hill, pages 11-13, (1996)).

The invention also includes pharmaceutical compositions comprising one or more of the compounds of the invention, or their salts or prodrugs forms thereof, with a pharmaceutically acceptable ingredient.

The pharmaceutical compositions can be prepared so that they may be administered orally, dermally, parenterally, nasally, ophthalmically, otically, sublingually, rectally or vaginally. Dermal administration includes topical application or transdermal administration. Parenteral administration includes intravenous, intraarticular, intramuscular, and subcutaneous injections, as well as use of infusion techniques. One or more compounds of the invention may be present in association with one or more non-toxic pharmaceutically acceptable ingredients and optionally, other active anti-proliferative agents, to form the pharmaceutical composition. These compositions can be prepared by applying known techniques in the art such as those taught in *Remington's Pharmaceutical Sciences* (Fourteenth Edition), Managing Editor, John E. Hoover, Mack Publishing Co., (1970) or *Pharmaceutical Dosage Form and Drug Delivery Systems* (Sixth Edition), edited by Ansel *et al.*, publ. by Williams & Wilkins, (1995).

Commonly used pharmaceutical ingredients which can be used as appropriate to formulate the composition for its intended route of administration include:

acidifying agents, examples include but are not limited to acetic acid, citric acid, fumaric acid, hydrochloric acid, nitric acid;

alkalinizing agents, examples include but are not limited to ammonia solution, ammonium carbonate, diethanolamine, monoethanolamine, potassium hydroxide, sodium borate, sodium carbonate, sodium hydroxide, triethanolamine, trolamine;

adsorbents, examples include but are not limited to powdered cellulose and activated charcoal;

aerosol propellants, examples include but are not limited to carbon dioxide, CCl_2F_2, F_2ClC-$CClF_2$ and $CClF_3$;

air displacement agents, examples include but are not limited to nitrogen and argon;

antifungal preservatives, examples include but are not limited to benzoic acid, butylparaben, ethylparaben, methylparaben, propylparaben, sodium benzoate;

antimicrobial preservatives, examples include but are not limited to benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate and thimerosal;

antioxidants, examples include but are not limited to ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorus acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde

sulfoxylate, sodium metabisulfite;

binding materials, examples include but are not limited to block polymers, natural and synthetic rubber, polyacrylates, polyurethanes, silicones and styrene-butadiene copolymers;

buffering agents, examples include but are not limited to potassium metaphosphate, potassium phosphate monobasic, sodium acetate, sodium citrate anhydrous and sodium citrate dihydrate;

carrying agents, examples include but are not limited to acacia syrup, aromatic syrup, aromatic elixir, cherry syrup, cocoa syrup, orange syrup, syrup, corn oil, mineral oil, peanut oil, sesame oil, bacteriostatic sodium chloride injection and bacteriostatic water for injection;

chelating agents, examples include but are not limited to edetate disodium and edetic acid;

colorants, examples include but are not limited to FD&C Red No. 3, FD&C Red No. 20, FD&C Yellow No. 6, FD&C Blue No. 2, D&C Green No. 5, D&C Orange No. 5, D&C Red No. 8, caramel and ferric oxide red;

clarifying agents, examples include but are not limited to bentonite;

emulsifying agents, examples include but are not limited to acacia, cetomacrogol, cetyl alcohol, glyceryl monostearate, lecithin, sorbitan monooleate, polyethylene 50 stearate;

encapsulating agents, examples include but are not limited to gelatin and cellulose acetate phthalate;

flavorants, examples include but are not limited to anise oil, cinnamon oil, cocoa, menthol, orange oil, peppermint oil and vanillin;

humectants, examples include but are not limited to glycerin, propylene glycol and sorbitol;

levigating agents, examples include but are not limited to mineral oil and glycerin;

oils, examples include but are not limited to arachis oil, mineral oil, olive oil, peanut oil, sesame oil and vegetable oil;

ointment bases, examples include but are not limited to lanolin, hydrophilic ointment, polyethylene glycol ointment, petrolatum, hydrophilic petrolatum, white ointment, yellow ointment, and rose water ointment;

penetration enhancers (transdermal delivery), examples include but are not limited to monohydroxy or polyhydroxy alcohols, saturated or unsaturated fatty alcohols, saturated or unsaturated fatty esters, saturated or unsaturated dicarboxylic acids, essential oils, phosphatidyl derivatives, cephalin, terpenes, amides, ethers, ketones and ureas;

plasticizers, examples include but are not limited to diethyl phthalate and glycerin;

s **lvents**, examples include but are not limited to alcohol, corn oil, cottonseed oil, glycerin, isopropyl alcohol, mineral oil, oleic acid, peanut oil, purified water, water for injection, sterile water for injection and sterile water for irrigation;

stiffening agents, examples include but are not limited to cetyl alcohol, cetyl esters wax, microcrystalline wax, paraffin, stearyl alcohol, white wax and yellow wax;

suppository bases, examples include but are not limited to cocoa butter and polyethylene glycols (mixtures);

surfactants, examples include but are not limited to benzalkonium chloride, nonoxynol 10, oxtoxynol 9, polysorbate 80, sodium lauryl sulfate and sorbitan monopalmitate;

suspending agents, examples include but are not limited to agar, bentonite, carbomers, carboxymethylcellulose sodium, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, kaolin, methylcellulose, tragacanth and veegum;

sweetening agents, examples include but are not limited to aspartame, dextrose, glycerin, mannitol, propylene glycol, saccharin sodium, sorbitol and sucrose;

tablet anti-adherents, examples include but are not limited to magnesium stearate and talc;

tablet binders, examples include but are not limited to acacia, alginic acid, carboxymethylcellulose sodium, compressible sugar, ethylcellulose, gelatin, liquid glucose, methylcellulose, povidone and pregelatinized starch;

tablet and capsule diluents, examples include but are not limited to dibasic calcium phosphate, kaolin, lactose, mannitol, microcrystalline cellulose, powedered cellulose, precipitated calcium carbonate, sodium carbonate, sodium phosphate, sorbitol and starch;

tablet coating agents, examples include but are not limited to liquid glucose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, ethylcellulose, cellulose acetate phthalate and shellac;

tablet direct compression excipients, examples include but are not limited to dibasic calcium phosphate;

tablet disintegrants, examples include but are not limited to alginic acid, carboxymethylcellulose calcium, microcrystalline cellulose, polacrillin potassium, sodium alginate, sodium starch glycollate and starch;

tablet glidants, examples include but are not limited to colloidal silica, corn starch and talc);

tablet lubricants (examples include but are not limited to calcium stearate, magnesium stearate, mineral oil, stearic acid and zinc stearate;

tablet/capsule opaquants, examples include but are not limited to titanium dioxide;

tablet polishing agents, examples include but are not limited to carnuba wax and white wax;

thickening agents, examples include but are not limited to beewax, cetyl alcohol and paraffin;

tonicity agents, examples include but are not limited to dextrose and sodium chloride;

viscosity increasing agents, examples include but are not limited to alginic acid, bentonite, carbomers, carboxymethylcellulose sodium, methylcellulose, povidone, sodium alginate and tragacanth; and

wetting agents, examples include but are not limited to heptadecaethylene oxycetanol, lecithins, polyethylene sorbitol monooleate, polyoxyethylene sorbitol monooleate, and polyoxyethylene stearate.

Depending on the route of administration, the compositions can take the form of aerosols, capsules, creams, elixirs, emulsions, foams, gels, granules, inhalants, lotions, magmas, ointments, peroral solids, powders, sprays, syrups, suppositories, suspensions, tablets and tinctures.

The therapeutic methods of the invention generally comprise administering to a subject in need thereof, a pharmaceutically effective amount of a compound. The compounds of the invention can be administered in a amount effective to inhibit the activity of a kinase, e.g., a tyrosine kinase, such as src kinase. The compounds of the invention can also be administered in a "growth inhibitory amount," i.e., an amount of the compound which is pharmaceutically effective to inhibit or decrease proliferation of target cells. The compounds can also be administered in a "differentiation modulating amount", e.g., "differentiation-inducing amount" or "differentiation-inhibiting amount," which is an amount of the compound which is pharmaceutically effective to modulate differentiation of target cells. The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

Toxicity and therapeutic efficacy of the compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such reagents to the site of affected tissue in order to minimize potential damage to normal cells and, thereby, reduce side effects.

Data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such reagents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any reagent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Based on these assays, it is possible to derive an appropriate dosage for administration to subjects by combining IC_{50} data with appropriate pharmacokinetic evaluation.

Pharmaceutical compositions containing a compound of the invention may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose, sodium crosscarmellose, corn

starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropylmethyl-cellulose or hydroxypropylcellulose, or a time delay material such as ethyl cellulose, cellulose acetate buryrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions

may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the compound of the invention in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

Pharmaceutical compositions may be in the form of sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution ethanol, cremophore and isotonic sodium chloride solution.

Sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the compound of the invention is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulation.

The injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous

intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of the invention may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of the invention can be employed. For purposes of this application, topical application shall include mouth washes and gargles.

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will preferably be continuous rather than intermittent throughout the dosage regimen.

The compounds of the invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. The compounds may be administered simultaneously or sequentially. For example, the instant compounds may be useful in combination with known anti-cancer and

cytotoxic agents. Similarly, the instant compounds may be useful in combination with agents that are effective in the treatment and prevention of osteoporosis, inflammation, neurofibromatosis, restinosis, and viral infections. The instant compounds may also be useful in combination with inhibitors of other components of signaling pathways of cell surface growth factor receptors.

Drugs can be co-administered to a subject being treated with a compound of the invention include antineoplastic agents selected from vinca alkaloids, epipodophyllotoxins, anthracycline antibiotics, actinomycin D, plicamycin, puromycin, gramicidin D, taxol, colchicine, cytochalasin B, emetine, maytansine, or amsacrine. Methods for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk Reference" (PDR), e.g., 1996 edition (Medical Economics Company, Montvale, N.J. 07645-1742, USA).

Optional anti-proliferative agents that can be added to the composition include but are not limited to compounds listed on the cancer chemotherapy drug regimens in the 11th Edition of the Merck Index, (1996), which is hereby incorporated by reference, such as asparaginase, bleomycin, carboplatin, carmustine, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, doxorubicin (adriamycine), epirubicin, etoposide, 5-fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, irinotecan, leucovorin, lomustine, mechlorethamine, 6-mercaptopurine, mesna, methotrexate, mitomycin C, mitoxantrone, prednisolone, prednisone, procarbazine, raloxifen, streptozocin, tamoxifen, thioguanine, topotecan, vinblastine, vincristine, and vindesine.

Other anti-proliferative agents suitable for use with the composition of the invention include but are not limited to those compounds acknowldeged to be used in the treatment of neoplastic diseases in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Ninth Edition), editor Molinoff *et al.*, publ. by McGraw-Hill, pages 1225-1287, (1996), such as aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine cladribine, busulfan, diethylstilbestrol, 2', 2'-difluorodeoxycytidine, docetaxel, erythrohydroxynonyladenine, ethinyl estradiol, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, fludarabine phosphate, fluoxymesterone, flutamide, hydroxyprogesterone caproate, idarubicin,

interferon, medroxyprogesterone acetate, megestrol acetate, melphalan, mitotane, paclitaxel, pentostatin, N-phosphonoacetyl-L-aspartate (PALA), plicamycin, semustine, teniposide, testosterone propionate, thiotepa, trimethylmelamine, uridine, and vinorelbine.

Other anti-proliferative agents suitable for use with the composition of the invention include but are not limited to other anti-cancer agents such as epothilone, irinotecan, raloxifen and topotecan.

For all regimens of use disclosed herein for the invention, the daily oral dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The daily dosage for administration by injection, including intravenous, intramuscular, subcutaneous and parenteral injections, and use of infusion techniques will preferably be from 0.01 to 200 mg/kg of total body weight. The daily rectal dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The daily vaginal dosage regimen will preferably be from 0.01 to 200 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 to 200 mg administered between one to four times daily. The transdermal concentration will preferably be that required to maintain a daily dose of from 0.01 to 200 mg/kg. The daily inhalation dosage regimen will preferably be from 0.01 to 100 mg/kg of total body weight.

It will be appreciated by those skilled in the art that the particular method of administration will depend on a variety of factors, all of which are considered routinely when administering therapeutics. It will also be understood, however, that the specific dose level for any given patient will depend upon a variety of factors, including, but not limited to the activity of the specific compound employed, the age of the patient, the body weight of the patient, the general health of the patient, the gender of the patient, the diet of the patient, time of administration, route of administration, rate of excretion, drug combinations, and the severity of the condition undergoing therapy. It will be further appreciated by one skilled in the art that the optimal course of treatment, i.e., the mode of treatment and the daily number of doses of a compound of formulae (I) or (II) or a pharmaceutically acceptable salt thereof given for a defined number of days, can be ascertained by those skilled in the art using conventional treatment tests.

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with a compound of the invention to treat a disease, e.g., cancer.

When a composition according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms.

Kits of the invention

In one embodiment, compounds of the invention and/or materials and reagents required for administering the compounds of the invention may be assembled together in a kit. When the components of the kit are provided in one or more liquid solutions, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being particularly preferred.

The kit may further comprise one or more other drugs, e.g., chemo- or radiotherapeutic agent. These normally will be a separate formulation, but may be formulated into a single pharmaceutically acceptable composition. The container means may itself be geared for administration, such as an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the formulation may be applied to an infected area of the body, such as the lungs, or injected into an animal, or even applied to and mixed with the other components of the kit.

The compositions of these kits also may be provided in dried or lyophilized forms. When reagents or components are provided as a dried form, reconstitution generally is by the addition of a suitable solvent. It is envisioned that the solvent also may be provided in another container means. The kits of the invention may also include an instruction sheet defining administration of the agent and, e.g., explaining how the agent will decrease proliferation of cells.

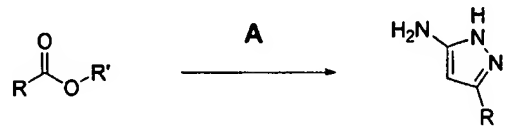
The kits of the present invention also will typically include a means for containing the vials in close confinement for commercial sale such as, e.g., injection or blow-molded plastic containers into which the desired vials are retained. Irrespective of the number or type of containers, the kits of the invention also may comprise, or be packaged with a separate instrument for assisting with the injection/administration or placement of the ultimate complex composition within the body of an animal. Such an instrument may be an inhalant, syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved

delivery vehicle. Other instrumentation includes devices that permit the reading or monitoring of reactions.

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

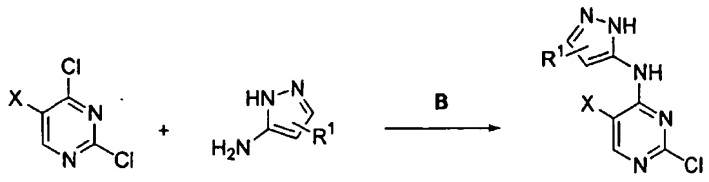
Examples 1-35

General Method A. Preparation of 5-amino-3-substituted pyrazoles



To a mixture of NaH (2.1 equiv) and THF (0.15 M) is added CH_3CN (2.1 equiv) and the required ester (1 equiv). The suspension is stirred at 65 °C for 16 h. The reaction is then quenched with an alcohol such as EtOH at 0 °C. Volatiles are evaporated and water added to the residue. This solution is cooled to 0 °C and the pH adjusted to ~3 with conc. HCl. The solution is extracted with Et_2O (3x) to give the crude β-ketonitrile intermediate. The crude β-ketonitrile (1 equiv) is treated with EtOH (0.3 M) and hydrazine hydrate (1.3 equiv) and stirred at 70 °C for 15 h. Volatiles are evaporated and the crude residue is purified by flash column chromatography (1/9 $MeOH/CH_2Cl_2$) to give the required pyrazole whose structure is confirmed by LC/MS and ^1H NMR.

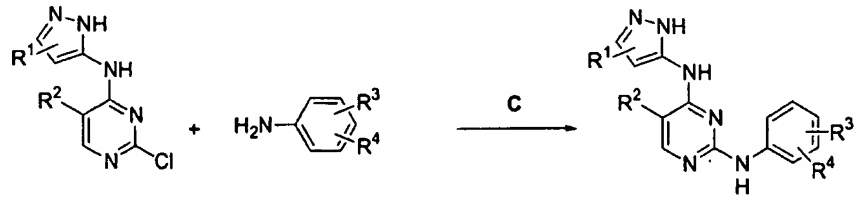
General Method B. Coupling of 5-amino-3-substituted pyrazoles with 5-substituted-2,4-dichloropyrimides



A solution of 5-substituted-2,4-dichlorpyrimidine (1 equiv), KOAc (1.3 equiv) and 5-amino-

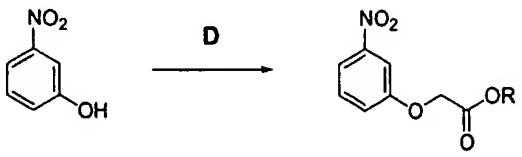
3-substituted pyrazole (1.1 equiv) in THF/H_2O (2/1, 0.15 M) is heated at 40 °C for 24 h. The reaction mixture is allowed to cool to rt, dissolved in EtOAc and washed with aqueous $NaHCO_3$. The combined organic layers are dried ($MgSO_4$) and concentrated under reduced pressure. The resulting crude solid is purified either by silica gel column chromatography or washing with other solvents to afford the *N*-(3-substituted-1*H*-pyrazol-5-yl)-2-chloro-5-substituted-4-pyrimidinamine intermediate whose structure is confirmed by LC/MS and ^1H NMR.

General Method C. Coupling of substituted anilines with *N*-(3-substituted-1*H*-pyrazol-5-yl)-2-chloro-5-substituted-4-pyrimidinamines



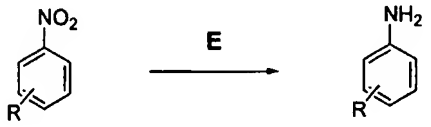
A solution of *N*-(3-substituted-1*H*-pyrazol-5-yl)-2-chloro-5-substituted-4-pyrimidinamine (1 equiv) and a substituted aniline (1 equiv) in an alcohol such as *n*-BuOH (0.08 M) with a catalytic amount of conc. HCl is heated at 100 °C for 24 h. The reaction is cooled to rt then concentrated under reduced pressure. The crude residue is dissolved in CH_2Cl_2 and washed with aqueous $NaHCO_3$. The combined organic layers are dried ($MgSO_4$) and concentrated under reduced pressure. Preparative thin-layer silica gel chromatography, silica gel column chromatography, and/or preparative HPLC are used to purify final products. LC/MS and ^1H NMR spectroscopy are used to confirm the structures of the final 2,4-substituted pyrimidinediamines.

General Method D. Preparation of 3-nitrophenoxyacetates



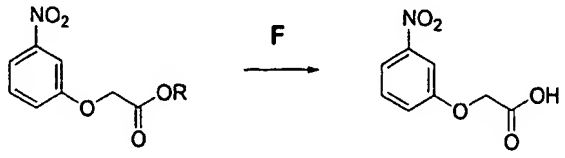
To a cooled solution (0 °C) of 3-nitrophenol (1 equiv) in DMF (1.8 M) is added NaH (1 equiv) portionwise. The reaction mixture is allowed to stir 10 min and bromoacetate (1 equiv) is added dropwise via syringe. The reaction is allowed to stir, gradually warming to room temperature overnight. The mixture is placed in an ice bath and quenched with water then poured into a separatory funnel and extracted with EtOAc (3x). The organic layers are combined and washed with aq $NaHCO_3$ (1x), water (1x), brine (1x), dried ($MgSO_4$), filtered and concentrated. The brown residue is purified by silica gel chromatography (2/3 EtOAc/Hex) to furnish the intermediate 3-nitrophenoxyacetate compound as a yellow oil whose structure is confirmed by LC/MS and ^1H NMR.

General Method E. Hydrogenation of substituted nitrobenzenes to substituted anilines



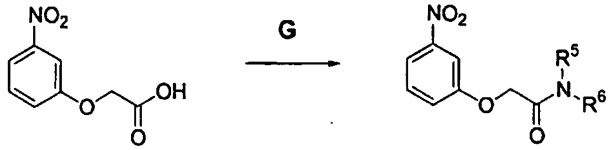
A solution of the substituted nitrobenzene (1 equiv) in ethanol (0.2 M) is added via syringe to a flask containing palladium on carbon (10 mol%). The reaction vessel is fitted with a balloon adapter and charged with hydrogen and evacuated three times until the reaction is under a H_2 atmosphere. The reaction is allowed to stir overnight and then purged with Ar and evacuated three times until an Ar atmosphere had been achieved. The reaction solution is filtered through a pad of Celite and washed with copious amounts of ethanol. The filtrate is concentrated in vacuo to afford the desired aniline whose structure is confirmed by LC/MS and ^1H NMR.

General Method F. Hydrolysis of 3-nitrophenoxyacetates to 3-nitrophenoxyacetic acid



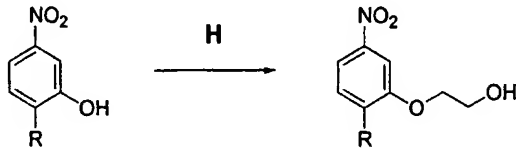
The 3-nitrophenoxyacetate intermediate (1 equiv) is hydrolyzed by stirring in 4N HCl in dioxane (3 equiv) at room temperature for 72 h. The reaction mixture is concentrated and triturated with Et_2O to yield 3-nitrophenoxyacetic acid as a white solid, whose structure is confirmed by LC/MS and ^1H NMR.

General Method G. Preparation of *N,N*-disubstituted 3-nitrophenoxyacetamides from 3-nitrophenoxyacetic acid



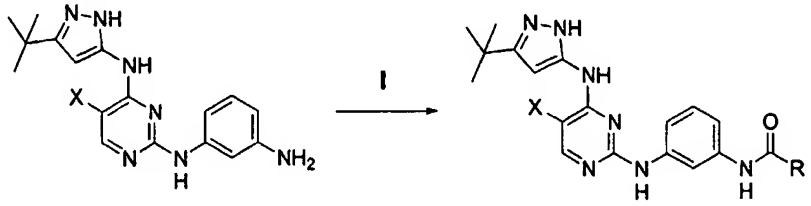
3-nitrophenoxyacetic acid (1 equiv) is dissolved in THF (0.25 M) and 1,1-carbonyldiimidazole (1 equiv) is added. The reaction mixture is stirred at rt for 3 h and then the desired amine (1.5 equiv) is added and the reaction is stirred overnight. The mixture is concentrated and purified by silica flash chromatography (1/1 EtOAc/Hex) to furnish the desired *N,N*-substituted 3-nitrophenoxyacetamide intermediate whose structure is confirmed by LC/MS and ^1H NMR.

General Method H. Preparation of 2-substituted-5-nitrophenoxyethanols from 2-substituted-5-nitrophenols



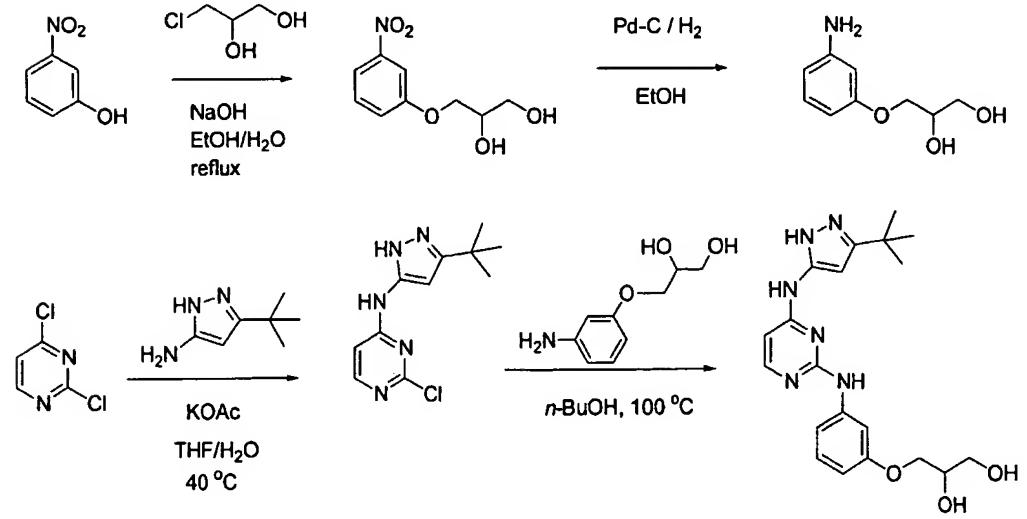
A mixture of the 2-substituted-5-nitrophenol (1 equiv) and ethylene carbonate (2 equiv) is heated to 190 °C for 24 h. The slightly unstable crude residue is cooled to rt and immediately purified by silica gel column chromatography (70/30 Hex/EtOAc) to afford the desired 2-substituted-5-nitrophenoxyethanol intermediate whose structure is confirmed by LC/MS and ^1H NMR.

General Method I. Preparation of *N*-[3-({4-[(3-tert-butyl-1*H*-pyrazol-5-yl)amino]-5-substituted-2-pyrimidinyl}amino)phenyl]substituted amides from N^2-(3-aminophenyl)-N^4-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-5-susbstitued-2,4-pyrimidinediamines



A mixture of the 3-aminophenyl advanced intermediate (1 equiv) and an acid chloride (1.5 equiv) in pyridine (0.15 M) is stirred at room temperature overnight. The mixture is concentrated in vacuo and purified by silica gel column chromatography (70/30-0/100 Hex/EtOAc) to afford the aminophenyl substituted amide whose structure is confirmed by LC/MS and ^{1}H NMR.

Example 1: Preparation of 3-[3-({4-[(3-tert-butyl-1H-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]-1,2-propanediol



To a solution of 3-nitrophenol (1.0 g, 7.19 mmol) in EtOH (5 ml) was added NaOH (359 mg, 8.99 mmol) dissolved in H_2O (1.5 ml). The reaction mixture was heated to reflux for 10 min and 3-chloro-1,2-propanediol (0.72 ml, 8.63 mmol) was added via syringe. The

reaction was allowed to reflux for 3 h then cooled and allowed to stir at room temperature overnight. The mixture was concentrated, dissolved in EtOAc and transferred to a separatory funnel and washed with 1N NaOH (1x), brine (1x), dried ($MgSO_4$), filtered and concentrated. The crude residue was purified by medium pressure silica gel chromatography (1/1 EtOAc/Hex) to furnish 948 mg (62%) of 3-(3-nitrophenoxy)-1,2-propanediol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79-7.76 (m, 2H), 7.58-7.48 (m, 1H), 7.39-7.35 (m, 1 H), 4.10-4.04 (m, 3H), 3.90-3.70 (m, 2H); MS (ESI-MS) 214 $[M+H]^+$.

A solution of 3-(3-nitrophenoxy)-1,2-propanediol (948 mg, 4.45 mmol) in EtOH (65 ml) was added via syringe to a flask containing palladium on carbon (10 mol%, 95 mg). The reaction vessel was fitted with a balloon adapter and charged with hydrogen and evacuated three times until the reaction was under a H_2 atmosphere. The reaction was allowed to stir overnight and then purged with Ar and evacuated three times until an Ar atmosphere had been achieved. The reaction solution was filtered through a pad of Celite and washed with copious amounts of ethanol. The filtrate was concentrated in vacuo to afford 800 mg (98%) of 3-(3-aminophenoxy)-1,2-propanediol. ^{1}H NMR (300 MHz, CD_3OD) δ 6.99-6.95 (m, 1H), 6.33-6.30 (m, 3H), 3.99-3.89 (m, 3 H), 3.67-3.58 (m, 2H); MS (ESI-MS) 184 $[M+H]^+$.

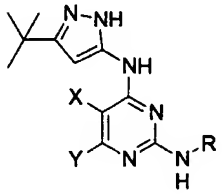
A solution of 2,4-dichloropyrimidine (11.92 g, 80.0 mmol), KOAc (9.42 g, 96.0 mmol, 1.2 equiv) and 5-amino-3-*tert*-butylpyrazole (11.14 g, 80.0 mmol) in THF/H_2O (225 mL, 2/1) was heated at 45 °C for 24 h. The reaction mixture was allowed to cool to rt, dissolved in EtOAc (200 mL) and washed with aq $NaHCO_3$ (2 x 200 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The resulting crude solid was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1/19) to give 8.62 (43%) of *N*-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-2-chloro-4-pyrimidinamine. ^{1}H NMR (300 MHz, DMSO) δ 12.2 (s, 1H), 10.3 (s, 1H), 8.16 (s, 1 H), 1.26 (s, 9H); MS (ESI-MS) 252 $[M+H]^+$. t_R 2.20 min (10-90% CH_3CN/H_2O).

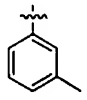
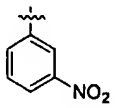
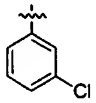
A solution of *N*-(3-*tert*-butyl-1*H*-pyrazol-5-yl)-2-chloro-4-pyrimidinamine (50 mg, 0.199 mmol) and 3-(3-aminophenoxy)-1,2-propanediol (36 mg, 0.199 mmol) in *n*-BuOH (2 ml) with one drop conc. HCl was placed in an 8 ml vial, capped and heated at 100 °C for 24 h in a shaker block. The reaction was cooled to rt and concentrated and the resulting crude oil was dissolved in MeOH (1.5 mL) and purified by preparative HPLC (10-90%CH_3CN/H_2O). The desired fractions were combined, concentrated, dissolved in CH_2Cl_2 and washed with aq $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and

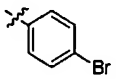
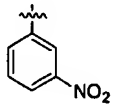
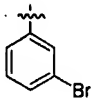
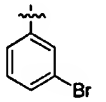
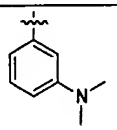
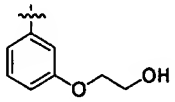
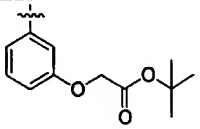
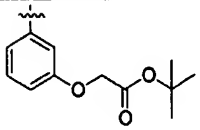
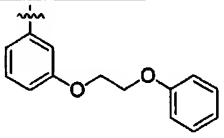
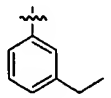
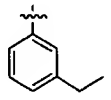
concentrated under reduced pressure to afford 16.6 mg (21%) of 3-[3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]-1,2-propanediol as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.94-7.93 (m, 1H), 7.29 (br s, 1H), 7.18-7.15 (m, 2H), 6.61-6.58 (m, 1H), 6.38-6.24 (m, 1H), 4.08-3.94 (m, 3H), 3.71-3.62 (m, 2H), 1.32 (s, 9H); MS (ESI-MS) 399 $[M+H]^+$; HPLC t_R 1.95 min (10-90% CH_3CN/H_2O).

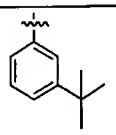
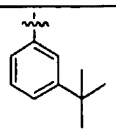
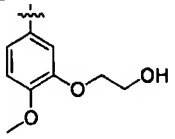
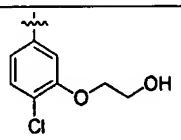
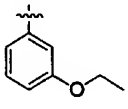
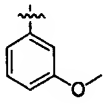
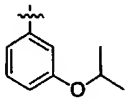
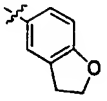
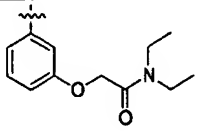
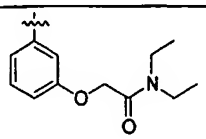
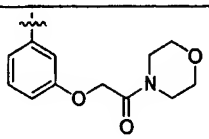
The compounds of examples 2-26 were prepared by general method C where a heterocyclic substituted pyrimidine (prepared by general methods A and B) is reacted with an aniline sidechain (commercially available or prepared by general methods D-I):

Table 1. Compounds Prepared by General Methods B and C.



Example #	X	Y	R	Aniline Sidechain Preparation	Characterization[a]
2	H	H		Commercial (ALDRICH)	$[M+H]^+$ 323 t_R 2.21 min.[b]
3	H	H		Commercial (ALDRICH)	$[M+H]^+$ 354 R_f = 0.16 (3/2 EtOAc/Hex)
4	H	H		Commercial (ALDRICH)	$[M+H]^+$ 323 R_f = 0.43 (7/3 EtOAc/Hex)

5	H	H		Commercial (ALDRICH)	$[M+H]^+$ 387 R_f = 0.25 (95/5 CH_2Cl_2/MeOH)
6	F	H		Commercial (ALDRICH)	$[M+H]^+$ 371 R_f = 0.44 (3/2 EtOAc/Hex)
7	H	H		Commercial (ALDRICH)	$[M+H]^+$ 387 R_f = 0.20 (95/5 CH_2Cl_2/MeOH)
8	F	H		Commercial (ALDRICH)	$[M+H]^+$ 405 R_f = 0.41 (2/3 EtOAc/Hex)
9	H	H		Commercial (ALDRICH)	$[M+H]^+$ 352 t_R 1.75 min.[b]
10	H	H		H,E	$[M+H]^+$ 368 R_f = 0.17(EtOAc)
11	F	H		D,E	$[M+H]^+$ 457 t_R 2.60 min.[b]
12	H	H		D,E	$[M+H]^+$ 439 t_R 2.57 min.[a]
13	H	H		Commercial (SALOR)	$[M+H]^+$ 445 t_R 2.50 min.[b]
14	F	H		Commercial (ALDICH)	$[M+H]^+$ 355 t_R 2.56 min.[b]
15	H	H		Commercial (ALDRICH)	$[M+H]^+$ 337 t_R 2.45 min.[b]

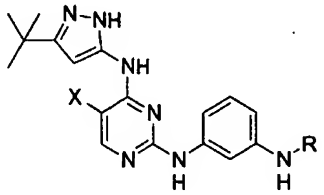
16	F	H		Commercial (MAYBRIDGE)	$[M + H]^+$ 383 t_R 2.67 min.[b]
17	H	H		Commercial (MAYBRIDGE)	$[M + H]^+$ 365 t_R 2.57 min.[b]
18	H	H		H,E	$[M + H]^+$ 399 t_R 1.62 min.[b]
19	H	H		H,E	$[M + H]^+$ 403 R_f = 0.25 (EtOAc)
20	H	H		Commercial (ALDRICH)	$[M + H]^+$ 353 t_R 2.27 min.[b]
21	H	H		Commercial (ALDRICH)	$[M + H]^+$ 339 R_f = 0.18 (95/5 CH_2Cl_2/MeOH)
22	H	H		Commercial (MAYBRIDGE)	$[M + H]^+$ 367 t_R 2.39 min.[b]
23	H	H		Ref.[c]	$[M + H]^+$ 351 R_f = 0.26 (EtOAc)
24	H	H		F,G,E	$[M + H]^+$ 438 t_R 2.23 min.[b]
25	F	H		F,G,E	$[M + H]^+$ 456 t_R 2.91 min.[b]
26	H	H		F,G,E	$[M + H]^+$ 452 t_R 2.07 min.[b]

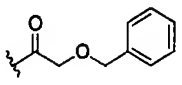
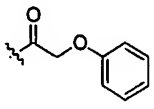
[a]The structures of the final compounds were confirmed by ^{1}H NMR spectroscopy and the spectra were consistent with the desired chemical structures.

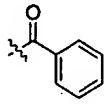
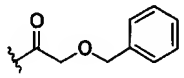
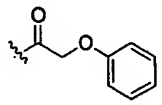
[b]Analytical HPLC were obtained using a Gilson HPLC equipped with a quaternary pump, a variable wavelength detector set at 254 nm, a YMC pro C-18 column (50 x 4.6 mm, 12μm). The eluents were A: acetonitrile w/0.1% TFA and B: H_2O w/0.1% TFA. Gradient elution from 10% B to 90% over 4 min at a flowrate of 4.0 mL/min was used with an initial hold of 0.5 min and a final hold at 90% B of 0.5 minutes. Total run time was 5 min.

[c]For preparation of 5-amino-2,3-dihydrobenzofurane see Mitchell, H.; Leblanc, Y. *J. Org. Chem.* **1994**, *59*, 682-687.

Table 2. Compounds Prepared by General Methods B, C, E and I.

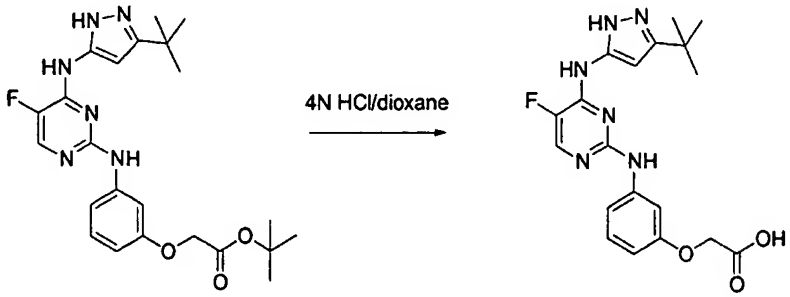


Example #	X	R	Characterization[a]
27	F	H	$[M + H]^+$ 342 R_f = 0.17 (94/6 CH_2Cl_2/MeOH)
28	H	H	$[M + H]^+$ 324 R_f = 0.57 (9/1 CH_2Cl_2/MeOH)
29	H		$[M + H]^+$ 472 R_f = 0.24 (94/6 CH_2Cl_2/MeOH)
30	H		$[M + H]^+$ 458 R_f = 0.35 (94/6

			CH_2Cl_2/MeOH)
31	H		$[M + H]^+$ 428 R_f = 0.18 (94/6 CH_2Cl_2/MeOH)
32	F		$[M + H]^+$ 490 R_f = 0.23 (94/6 CH_2Cl_2/MeOH)
33	F		$[M + H]^+$ 476 R_f = 0.12 (1/1 EtOAc/Hex)

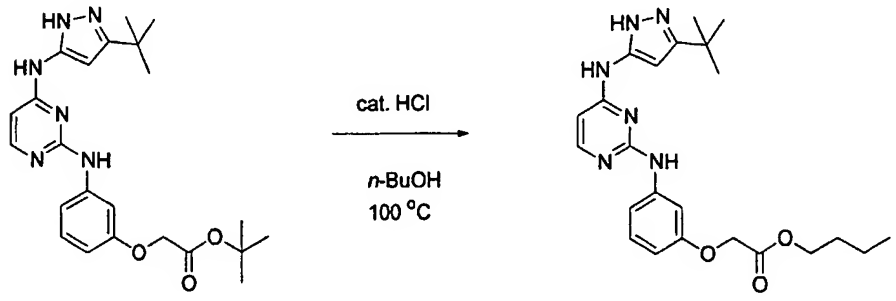
[a]The structures of the final compounds were confirmed by ^{1}H NMR spectroscopy and the spectra were consistent with the desired chemical structures.

Example 34: Preparation of [3-({4-[(3-tert-butyl-1H-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenoxy]acetic acid.



A solution of *tert*-butyl [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenoxy]acetate (1 equiv) in 4N HCl/Dioxane (3 equiv) was stirred at room temperature over 72 h. The mixture was concentrated and the crude solid was triturated in Et_2O, filtered, washed with Et_2O and dried in a hi-vac oven to afford [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-5-fluoro-2-pyrimidinyl}amino)phenoxy]acetic acid. MS (ESI-MS) 401 $[M + H]^+$; t_R 2.60 min (10-90% CH_3CN/H_2O).

Example 35: Preparation of butyl [3-({4-[(3-tert-butyl-1H-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]acetate.



A solution of *tert*-butyl [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]acetate (1 equiv), cat. conc. HCl in *n*-BuOH (0.30 M) was stirred at 100°C for 24 h. The mixture was concentrated and purified by preparative HPLC (10-90%CH_3CN/H_2O) to afford butyl [3-({4-[(3-*tert*-butyl-1*H*-pyrazol-5-yl)amino]-2-pyrimidinyl}amino)phenoxy]acetate. MS (ESI-MS) 439 $[M + H]^+$; t_R 2.59 min (10-90% CH_3CN/H_2O).

Assays for testing the activity of the compounds

This section describes assays that can be used to characterize compounds of the invention, e.g., src kinase activity assays; assays for testing the activity of compounds on kinases other than src; and assays for testing the activity of compounds on cell proliferation and differentiation.

A preferred method for measuring src kinase activity (a "src biochemical assay") uses ATP (5 μM/well) mixed with biotinylated poly-GAT substrate (10 nM/well), Streptavidin-APC (15 nM/well) and European-labeled anti-phosphotyrosine antibody (2.5 nM/well). 10 μl of a mixture of these components is added to each well of a black 96-well plate, with or without test compound (5 μl desired concentration of compound in DMSO). 75 μl of assay buffer (50 mM HEPES pH 7.5, 0.1 mM EDTA, 0.015% BRIJ 35 solution, 0.1 mg/mL BSA, 0.1% beta-mercaptoethanol, 10 mM magnesium chloride) is then added to each well. Last, the src kinase (0.1 units/well) (Upstate Biotech, Lake Placid, NY) is added (10 μl) to a final

volume of 100 µl. After 3-hour incubation at room temperature, plates are read on Wallac 1420 Victor Multilabel Counter (Perkin Elmer™ Life Sciences, Boston, MA) at 665 and 615 nm. A specific signal is the ratio of the value of the signal at 665 and the value of the signal at 615 multiplied by 10,000 (i.e., (signal at 665/signal at 615) x 10,000). Compounds that cause the specific signal to decrease inhibit the kinase activity of src. Percent inhibitions and/or IC_{50} values can then calculated based on specific signals from wells that have no compound added, i.e., zero percent inhibition.

A specific signal is the ratio of the value of the signal at 665 and the value of the signal at 615 multiplied by 10,000 (i.e., (signal at 665/signal at 615) x 10,000). Compounds that cause the specific signal to decrease inhibit the kinase activity of src. Percent inhibitions and/or IC_{50} values can then calculated based on specific signals from wells that have no compound added, i.e., zero percent inhibition.

Compounds of examples 1, 3, 14-15, 18-19, 27-30 and 33 show an IC_{50} less than 500 nM in the src biochemical assay. Compounds of examples 2, 4, 10, 20, 23-26 and 31-32 show an IC_{50} greater than 500 nM but less than 1.0 µM in the src biochemical assay. Compounds of examples 5-9, 11-13, 16-17, 21-22 and 34-35 show an IC_{50} greater than 1 µM and/or percent inhibition greater than 30 and less than 50 in the src biochemical assay.

It will be understood by a person of skill in the art that modified versions of the src biochemical assay described above can be conducted. These alternative assays can also be used to test the inhibitory activity of compounds of the invention or analogs or derivatives thereof.

The assay can also be adapted to determine the inhibitory activity of compounds towards kinases other than src kinases. For example, the src kinase enzyme in the above assay can be replaced with another kinase. When testing the inhibitory activity on kinases that are not tyrosine kinases, the antibody in the assay may also have to be replaced with an antibody that is specific for the phosphorylated residue, which has been phosphorylated by the kinase.

The effect of compounds on cell proliferation can be determined, e.g., by incubating cells with varying amounts of the compounds and counting the cells over time. Viable cells can be counted by staining the cells with a specific dye, e.g., Trypan Blue, according to methods well known in the art. Other methods include measuring the incorporation of a labeled molecule into DNA or RNA or protein of cells. For example, cell proliferation is

often measured by ^3H thymidine or 5-bromodeoxyuridine incorporation assays, also well known in the art. An increase in ^3H thymidine or 5-bromodeoxyuridine incorporation in cells incubated with a test compound that is similar to that in cells non incubated with the test compound indicates that the test compound is essentially not inhibiting the proliferation of the cells. On the contrary, a lower ^3H thymidine or 5-bromodeoxyuridine incorporation in cells incubated with a test compound relative to cells that were not treated with the test compound indicates that the test compound inhibits cell proliferation.

The effect of a compound on cell differentiation can be determined by visualization of the cells after having been contacted with the compound, preferably by comparison with cells which have not been contacted with the compound. The differentiation of certain cells is visible by the naked eye (e.g., that of 3T3L1 cells), whereas that of other cells may require the use of a microscope. Specific dyes can also be used to evaluate the state of differentiation of cells. Cell differentiation can also be monitored by measuring the expression level of certain genes, whose expression is known to vary during differentiation of the cells.

The effect of a compound on a cell can be determined in a cell that contains an abnormal kinase, e.g., a mutated kinase gene, or a cell which over-expresses a kinase. For example the cell can be a cell expressing a mutated form of a tyrosine kinase, e.g., src kinase, thereby transforming the cell. The cell can also be a cell that has an abnormal proliferation which is not caused by an abnormal activity or level of a kinase. Cells that can be used for testing compounds of the invention include cell lines and primary cell cultures. Numerous cell lines that are transformed, e.g., by over-expression of a proto-oncogene, which encodes, e.g., a kinase, are available, e.g., from the American Type Culture Collection (ATCC, 10801 University Blvd., Manassas, Virginia 20110. Cell lines over-expressing a gene, e.g., a kinase, can be prepared by transient, or preferably, stable transfection of cells with an expression plasmid containing the gene, according to methods well known in the art. Nucleic acids for use in transforming cells, e.g., nucleic acids encoding kinases, are also publicly available or can readily be obtained. Cell lines can also be obtained from transgenic animals, e.g., animals overexpressing a kinase or expressing a mutated kinase. For example, MG 1361 is a breast carcinoma cell line obtained from the MMTV-neu transgenic mouse (Sacco *et al.*, Breast Cancer Res. Treat., 47:171-180 (1998)). Primary cell cultures can be established from biopsies obtained from patients, e.g., patients having cancer.

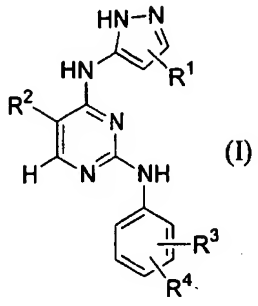
The present invention also provides methods of testing a compound (e.g., the candidate drug) for its inhibition of src, its antiproliferative effect, its effect on cell differentiation and/or its toxicity on normal or wild-type cells in animals, e.g., transgenic animals, e.g., mice. Transgenic mice are produced that express a transforming agent (e.g., a growth factor receptor) under the control of a promoter, e.g., a tissue specific promoter. Such mice develop carcinomas that have genetic and pathological features that closely resemble human cancers. For example, mice expressing viral polyoma middle T antigen under the control of the MMTV promoter produces highly metastatic mammary tumors with elevated c-src kinase activity (Guy *et al.* (1994) Genes and Dev. 8:23). Nude mice in which tumor cell lines have been administered can also be used. For example, breast cancer cell lines over-expressing c-src can be administered to nude mice (*see, e.g.,* Biscardi *et al.* (1998) Mol. Carcinog. 21: 261). The ability of the compound to inhibit tumor formation or growth is then ascertained. In one embodiment the size of the tumor is monitored by determining the tumor size and/or weight. The compounds can be administered by a variety of ways including orally, subcutaneously, or intraperitoneally. Generally, at least two groups of animals are used in the assay, with at least one group being a control group which is administered the administration vehicle without the compound.

An animal model for osteoporosis that can be used for testing the activity of compounds is described, e.g., in Missbach *et al.* (1999) Bone 24:437 and in Sims *et al.* (1999) J. Bone Miner. Res. 14: S183.

Other embodiments of the invention will be apparent to the skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

We claim:

1. A compound of the formula (I)



(I)

wherein

R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, adamantyl, phenyl, or a 5-membered heteroaromatic containing a single heteroatom selected from N, O, and S;

R^2 represents H, F, Cl, or C_{1-4} alkyl;

R^3 represents H, halogen, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl;

R^4 represents

halogen,

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

NO_2,

C_{1-6} alkyl,

NR^5R^6,

$O(CH_2)_{1-4}$-CO_2R^7,

$O(CH_2)_{1-4}$-$C(O)NR^5R^6$,

$N(R^5)C(O)CH_2OR^8$,

$OC(O)R^9$,

$C(O)NR^5R^6$,

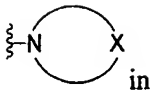
CO_2R^7, or

CN;

wherein

R^5 and R^6 each independently represents H or C_{1-4} alkyl; or

R^5 and R^6 may be joined, and taken together with the nitrogen atom to which they are attached, constitute a 5-6-membered nonaromatic heterocycle


in which X represents NR^5, O, S, or $C(R^5)_2$;

R^7 represents H, C_{1-6} alkyl, or phenyl;

R^8 represents H, phenyl, benzyl, or C_{1-6} alkyl;

R^9 represents C_{1-6} alkyl or phenyl; or

R^3 and R^4 are joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5;

or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein

R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, or phenyl.

3. A compound of claim 1 wherein

R^1 represents C_{1-6} alkyl or C_{3-6} cycloalkyl.

4. A compound of claim 1 wherein

R^1 represents C_{1-6} alkyl.

5. A compound of claim 1 wherein

R^2 represents H or F.

6. A compound of claim 1 wherein

R^3 represents H, Cl, F, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl.

7. A compound of claim 1 wherein

R^3 represents H, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl.

8. A compound of claim 1 wherein

R^3 represents H or $O(C_{1-4}$ alkyl).

9. A compound of claim 1 wherein

R^3 and R^4 are joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5.

10. A compound of claim 1 wherein

R^4 represents

halogen,

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

NO_2,

C_{1-6} alkyl,

NR^5R^6,

$O(CH_2)_{1-4}$-CO_2R^7,

$N(R^5)C(O)CH_2OR^8$,

$OC(O)R^9$, or

$C(O)NR^5R^6$.

11. A compound of claim 1 wherein

R^4 represents

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

C_{1-6} alkyl,

NR^5R^6,

$N(R^5)C(O)CH_2OR^8$, or

$C(O)NR^5R^6$.

12. A compound of claim 1 wherein

R^4 represents

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

NR^5R^6, or

$N(R^5)C(O)CH_2OR^8$.

13. A compound of claim 1 wherein

R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, or phenyl;

R^2 represents H, Cl, F, or C_{1-4} alkyl;

R^3 represents H, Cl, F, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl;

R^4 represents

halogen,

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

NO_2,

C_{1-6} alkyl,

NR^5R^6,

$O(CH_2)_{1-4}$-CO_2R^7,

$N(R^5)C(O)CH_2OR^8$,

$OC(O)R^9$, or

$C(O)NR^5R^6$, or

R^3 and R^4 are joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5.

14. A compound of claim 1 wherein

R^1 represents C_{1-6} alkyl or C_{3-6} cycloalkyl;

R^2 represents H or F;

R^3 represents H, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl;

R^4 represents

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

C_{1-6} alkyl,

NR^5R^6,

$N(R^5)C(O)CH_2OR^8$, or

$C(O)NR^5R^6$.

15. A compound of claim 1 wherein

R^1 represents C_{1-6} alkyl;

R^2 represents H or F;

R^3 represents H or $O(C_{1-4}$ alkyl); and

R^4 represents

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

NR^5R^6, or

$N(R^5)C(O)CH_2OR^8$.

16. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

17. A method of inhibiting Src kinase receptors in a subject comprising contacting said receptors with the compound according to claim 1.

18. A method for treating a disease associated with a src kinase in a subject, comprising administering to said subject a therapeutically effective amount of a compound according to claim 1, such that the disease is treated.

19. The method of claim 18 wherein said disease is cancer or osteoporosis.

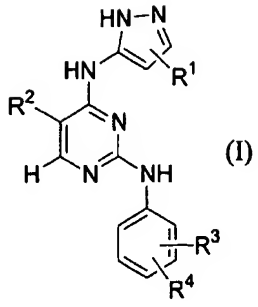
20. A method for treating cancer in a subject, comprising administering to said subject a therapeutically effective amount of a compound according to claim 1, such that the cancer is treated.

21. The method of claim 20, wherein the cancer is selected from the group consisting of breast cancer, colon cancer, pancreatic cancer, lung cancer, neural cancer, esophageal cancer, gastric cancer, melanoma and Kaposi's sarcoma.

22. A method for treating a non-malignant proliferative disease in a subject, comprising administering to said subject a therapeutically effective amount of a compound according to claim 1, such that the non-malignant proliferative disease is treated.

23. A method for treating osteoporosis in a subject, comprising administering to said subject a therapeutically effective amount of a compound according to claim 1, such that the osteoporosis is treated.

24. A method for making a compound of the formula (I)



(I)

wherein

R^1 represents C_{1-6} alkyl, C_{3-6} cycloalkyl, adamantyl, phenyl, or a 5-membered heteroaromatic containing a single heteroatom selected from N, O, and S;

R^2 represents H, F, Cl, or C_{1-4} alkyl;

R^3 represents H, halogen, $O(C_{1-4}$ alkyl), or C_{1-6} alkyl;

R^4 represents

halogen,

$O(C_{1-4}$ alkyl) optionally substituted by OH or phenoxy,

NO_2,

C_{1-6} alkyl,

NR^5R^6,

$O(CH_2)_{1-4}$-CO_2R^7,

$O(CH_2)_{1-4}$-$C(O)NR^5R^6$,

$N(R^5)C(O)CH_2OR^8$,

$OC(O)R^9$,

$C(O)NR^5R^6$,

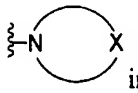
CO_2R^7, or

CN;

wherein

R^5 and R^6 each independently represents H or C_{1-4} alkyl; or

R^5 and R^6 may be joined, and taken together with the nitrogen atom to which they are attached, constitute a 5-6-membered nonaromatic heterocycle



in which X represents NR^5, O, S, or $C(R^5)_2$;

R^7 represents H, C_{1-6} alkyl, or phenyl;

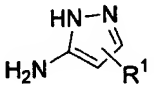
R^8 represents H, phenyl, benzyl, or C_{1-6} alkyl;

R^9 represents C_{1-6} alkyl or phenyl; or

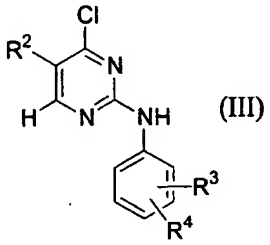
R^3 and R^4 are joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), $S(O)_2$, and NR^5,

comprising

coupling a compound of formula (II)

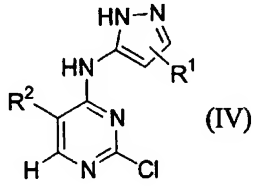

(II)

with a compound of formula (III)

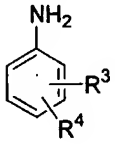

(III)

or

coupling a compound of formula (IV)


(IV)

with a compound of formula (V)


(V)

, to produce a compound of formula (I).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/30980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/506 C07D403/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 60816 A (AMGEN INC) 23 August 2001 (2001-08-23) page 72 -page 76; claim 1 page 25; compound 5 page 19, line 9 - line 11 ---	1-24
X	WO 97 19065 A (CELLTECH THERAPEUTICS LTD ;DAVIS PETER DAVID (GB); MOFFAT DAVID FE) 29 May 1997 (1997-05-29) page 77; claim 1 page 1, line 35 -page 2, line 4 ---	1-24
X	WO 01 64656 A (PEARSON STUART ERIC ;PEASE ELIZABETH JANET (GB); ASTRAZENECA UK LT) 7 September 2001 (2001-09-07) page 52 -page 54; claim 1 page 2, line 1 - line 9 ---	1-24
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 December 2002	17/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fink, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/30980

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 02 062789 A (MILLER ANDREW ;KNEGTEL RONALD (GB); BEBBINGTON DAVID (GB); CHARRIE) 15 August 2002 (2002-08-15) page 321 -page 323; claim 1 page 329; claim 11	1-24

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8 (all partly), 10 (partly), 13 (partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents halogen;

2. Claims: 1-8 (all partly), 10-24 (all partly)

the compounds of formula (I) wherein R4 represents O(C1-C4 alkyl) optionally substituted by OH or phenoxy;

3. Claims: 1-8 (all partly), 10 (partly), 13 (partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents NO2;

4. Claims: 1-8 (all partly), 10 (partly), 11 (partly), 13 (partly), 14 (partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents C1-C6 alkyl;

5. Claims: 1-8 (all partly), 10-24 (all partly)

the compounds of formula (I) wherein R4 represents NR5R6 or NR5C(O)CH2OR8;

6. Claims: 1-8 (all partly), 10 (partly), 13 (partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents O(CH2)1-4-CO2R7 or O(CH2)1-4-C(O)NR5R6;

7. Claims: 1-8 (all partly), 10 (partly), 13 (partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents OC(O)R9;

8. Claims: 1-8 (all partly), 10 (partly), 11 (partly), 13 (partly), 14 (partly), 16-24 (all partly)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the compounds of formula (I) wherein R4 represents C(O)NR5R6;

9. Claims: 1-8 (all partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents CO2R7;

10. Claims: 1-8 (all partly), 16-24 (all partly)

the compounds of formula (I) wherein R4 represents CN;

11. Claims: 1-8 (all partly), 9, 16-24 (all partly)

the compounds of formula (I) wherein R3 and R4 are joined to form a 5-6 membered nonaromatic heterocycle in which up to 2 ring members are selected from O, S, S(O), S(O)2, and NR5;

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/30980

Box I Observations where certain claims were found unsearchable (Continuati n of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 17-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 02/30980

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0160816 A	23-08-2001	AU	3704101 A	27-08-2001
		EP	1257546 A1	20-11-2002
		WO	0160816 A1	23-08-2001
		US	2002052386 A1	02-05-2002
WO 9719065 A	29-05-1997	AU	7631496 A	11-06-1997
		EP	0862560 A1	09-09-1998
		WO	9719065 A1	29-05-1997
		US	6235746 B1	22-05-2001
		US	5958935 A	28-09-1999
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		WO	0164656 A1	07-09-2001
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WO 02062789 A	15-08-2002	AU	3116602 A	01-07-2002
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		AU	9091201 A	26-03-2002
		AU	9091401 A	26-03-2002
		AU	9094401 A	26-03-2002
		AU	9101301 A	26-03-2002
		AU	9267001 A	26-03-2002
		AU	9455801 A	26-03-2002
		AU	9687101 A	26-03-2002
		AU	9687501 A	26-03-2002
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